

+1 Met Glu Ser Ile Ile Val Gly Ala Glu Thr Phe Gly Leu Ser Thr Ala Leu Glu Leu Ala Arg Asp Glu Tyr Lys Asn Ile Lys Cys

1 ATGGAGTCGA TAATTATACT TGGTGCCT ACTTTCGGC TTTCACAGG CTTACAGCTT GCCACAGATG GATACAGAA CATAAACGAA CATAAACGAT

.1 Phe Asp Lys Phe Pro Val Phe Ser Glu Ile Ala Ala Glu Asn Asp Ser Asn Lys Ile Phe His Tyr Asp Tyr Val Ala Pro Leu Ala Lys

91 TITGACAAGT TTCCGGTTCC ATCTGAGATA GCTGCTGGAA ACCGACACTAA CAAAGATTTT CACTACGATT ATGTGGCTCC CCTGGCTAA

.1 Pro Asn Ser Lys Glu Arg Leu Ser Leu Glu Ala Leu His Leu Tip Lys Thr Asp Pro Val Tyr Lys Pro Tyr His Pro Val Glu Phe

181 CCCATTCGA AGAACCGTT GAGTCCTCAA GCATTACGCC TTGGAGAGC AGATCCGGTG TACALACCGT ACTATCATCC GCTTAGGATT

.1 Ile Leu Ala Ala Ser Ser Asp Ala Pro Leu Leu His Asp Lys Glu Tyr Glu Glu Leu Glu Lys Asn Glu Leu Arg Asn Tyr Arg Tyr

271 ATCCTGGCTG CAAAGTCCCGA TGCTCATTAA AGGAACTACTA TGAAAGAGTTG CAAALAAACG GACTTCGCAA TTATCGTTAT

.1 Ile Ser Thr Pro Glu Glu Phe Arg Glu Tyr Leu Pro Ile Leu Lys Glu Pro Leu Pro Asn Tip Arg Glu Tyr Val Leu Asp Glu Asp Asn

361 ATTCAACTC CGGAGGATT TGCTGACTAT TTGCCCCATT TAAGGGCCCC GTTACCCAC TGCAAGAGAT ATGTTCTCGA CGAGATAAC

.1 Gly Tip Leu His Ala Arg Asp Ser Leu Lys Ser Ala Tyr Glu Glu Cys Lys Arg Leu Glu Val Glu Phe Val Asp Glu Asp Glu

451 GGATGGTGC ATGCTCGAGA CTCATGAA AGAGATGCCA AGGATGGGA GTGGGAAATTG TGTTGGAGA CGATGGGAA

.1 Ile Val Glu Leu Asn Glu Asn Glu Ile Arg Ala Arg Ser Glu Ala Ile Phe Ser Ala Glu Lys Tyr Val Leu Ser

541 ATTGTCGAAT TACTTAAACGA AAATGAAAG TTGACGGAA TTAGGCCAG ATCTGGCTGCC ATATTCTCGG CACAAAGATA TTGTTCTCAGC

.1 Ser Glu Ala Asn Ala Val Thr Leu Ile Asn Phe Glu Arg Glu Lys Glu Glu Lys Cys Phe Thr Leu Ala His Phe Lys Val Thr Asp Glu

631 TCTGGTCCAA ATGCGTAAAC GTTGTAAAT TTCCAGAGAC AGCTAGAGG TAAATGTTTC ACTTGGCAC ATTGTAAAGT GAAGGTGAA

.1 Glu Ala Lys Ala Phe Lys Ser Leu Pro Val Ile Phe Asn Ala Glu Lys Glu Phe Phe Glu Ala Asp Glu Asn Asn Glu Ile Lys Ile

721 GAAGCTAAAG CATTAAAG CTGGCGGTG CTTTCAATG CCGAAAGGG GTTITTTTC GAGGGTGTATG AAAATAACGA AAATCAAATT

.1 Cys Asn Glu Tyr Pro Glu Phe Thr Asn Glu Ser Glu Glu Ser Ile Pro Leu Tyr Arg Met Glu Ile Pro Leu Glu Ser Ala Leu

811 TGCAACGAGT ACCCTGGATT TACCCACACA ATGAAATCCG GAGAGTCTAT CCCACTCTAC CGGATGGAA TTCCACCTGA GTCAGGACTT

.1 Glu Ile Arg Glu Tyr Leu Lys Glu Thr Met Pro Glu Phe Ala Asp Arg Pro Phe Thr Lys Thr Arg Ile Cys Thr Asp Ser Pro

901 GAATTAGAC AATACTGAA AGAACCATG CCTCAGTTG CTGATAGACC TTTCACCAAG ACAAGAATTG GTTGGTGTAC CGACTCTCCC

.1 Asp Met Glu Ile Leu Cys Thr His Pro Glu Tyr Thr Asn Leu Ile Val Ala Ser Glu Asp Ser Glu Asn Ser Phe Lys Ile Met Pro

991 GACATGAAAT TGATCTGTG TACTAACCCA GAATACACCA ACCTTATTGT AGCATCGGGT GACTCTGGAA ATTCTGTTCAA GATCATGCCA

.1 Ile Ile Gly Lys Tyr Val Ser Lys Val Thr Lys Glu Asp Lys Glu Leu Asp Pro Glu Asp Lys Glu Cys Tip Lys Tip Arg Pro Glu

1081 ATCATGGCA ATATGTCAG CAAGGTTGT ACCAACGGTT ATAAGGATT GATACGCCAA GATACGCCAA GATACGCCAA GATACGCCAA GATACGCCAA

.1 Thr Tip Asp Lys Arg Glu Val Arg Tip Glu Glu Arg Tyr Val Ala Asp Leu Asn Glu Ile Glu Tip Val Ser Val Glu Asn

1171 ATCTGGACAA AGGGGGGCA GGTCGGCTGG GGTGGCTCGAT ACCGTGTTCGAC GATTTGAGC GATTTGAGC ATGGGTTTC TTGTTAAAT

.1 Pro Thr Pro His Leu Glu ...

1261 CCCACACAC ACCAACTAGA ATAA

FIG. 1

mesiiivgagtfglstatqlardgyknikcfdkfpvpseiaagndsnkifhydyvaplaknskerlslealhlwktdpvykpyyhp
 vgflaasssdapllhdkeyyeelqknqlrnyryistpeefreylpilkgp1pnwrgyvldgdngwlhardslksayeeckrlgvefv
 fgddgeivellnengkltgirarsgaissaqkyvlssganavtllnfqrqlegkcfelahfkvtdeakafkslpvlfnakegffffe
 adenenneikiicneypgfthtnesgesiplyrmeiplesaleirqylketmpqfadrpftktricwcldspdmqlilcthpeytlnliva
 sgdsignsfkimpriegkyvskvtkgdkglpedkecwkrpetwdkrqvrwggyrvadlneieewvsvenptphkle

FIG. 2

5' -atggagtcgataattatagttggtgccggactttgggcttccacagccitacagctgccagagatggatacaagaacataaaatgtttgacaagttccggtt
 ccatctgagatagctgcggaaacgacagtaacaagatttcactacgattatgtgcctccctggctaaacccaattcaaagaacggttgagtcgcgaagcattacac
 ctttggagacagatccggtgtacaaaccgtactatcatccggtaggattatcctggctgcgaatgtccattactgcatgataaggaataactatgaagagttg
 caaaaaaaacggacttcgaattatcgatattcaactcccgaggagttctgtgagtttgcccatttaaaggccccgttacccaactggagaggatatttcgcacg
 gagataacggatgggtgcatgcgcagactatgaaaagtgcatacgaagaatgcacaaacgcattggagttggatgttttttttttttttttttttttttttttttttt
 acttaacgaaaatggaaagtgcacggaaattagggccagatctggfcccataatctcggcacaaaaatatgttcagctctggfgc当地atgcacgttgtttaattt
 ccagagacagctagaaggtaatgtttcactttgcacattcaaaatgtgacggatgacggatgacggatgacggatgacggatgacggatgacggatgacggat
 ttttttcgaggctgatgaaaatacggaaatcaaaatgtcaacgcgatccctggatttacccacacaaatgaaatccggagactatccactctacggatggagattc
 cactcgagtcgcacttgcataatgcacccatgcctcgcattttgcgcataatgcacccatgcctcgcattttgcgcataatgcacccatgcctcgcataatgc
 tcaatgtatgttgcactcaccgcataacaccaacccatgttagcatcggttgactctggaaatcgatcaatgcacccatgcctcgcataatgcacccatgc
 tggatccaaaggatgatggatccggaaagataaagaatgcggaaatggcgctctggatccggatgggacaagcggggcagggtccctgggggtggatc
 ctgtttgcggatttgcacggaaatgtacggatggatccggatccggatccggatccggatccggatccggatccggatccggatccggatccggatccggatcc

FIG. 3

FAO-F1 5'-GGXACXTGGGXWSXWSXACXGCXYTXCA-3'
FAO-R2 3'-TCYTCRTYXGGYTCVAWRAARAAXCC-5'
in which * S=C+G Y=C+T R=A+G X=A+C+G+T W=A+T V=A+C+G

FAO-F3 : 5'-ATTCAAAGTGACGGATGAAGAAGCTAAAG-3'
adaptor primer : 3'-CGCAGTTCCCAGTCACGAC-5'

FAO-F5 5'-GTGCATACGAAGAACATGCAAACGATTGGGAGTGG-3'

FAO-R6 3'-CCATCCGTTATCTCCGTCGAGAACATATCCTC-5'

FAO-NcoI : 5'-ATCACCATGGAGTCGATAATTATAGTTGG-3'

FAO-XbaI : 3'-TTGATTCTAGACATGTATGTTGTAATCTTG-5'

FIG. 4

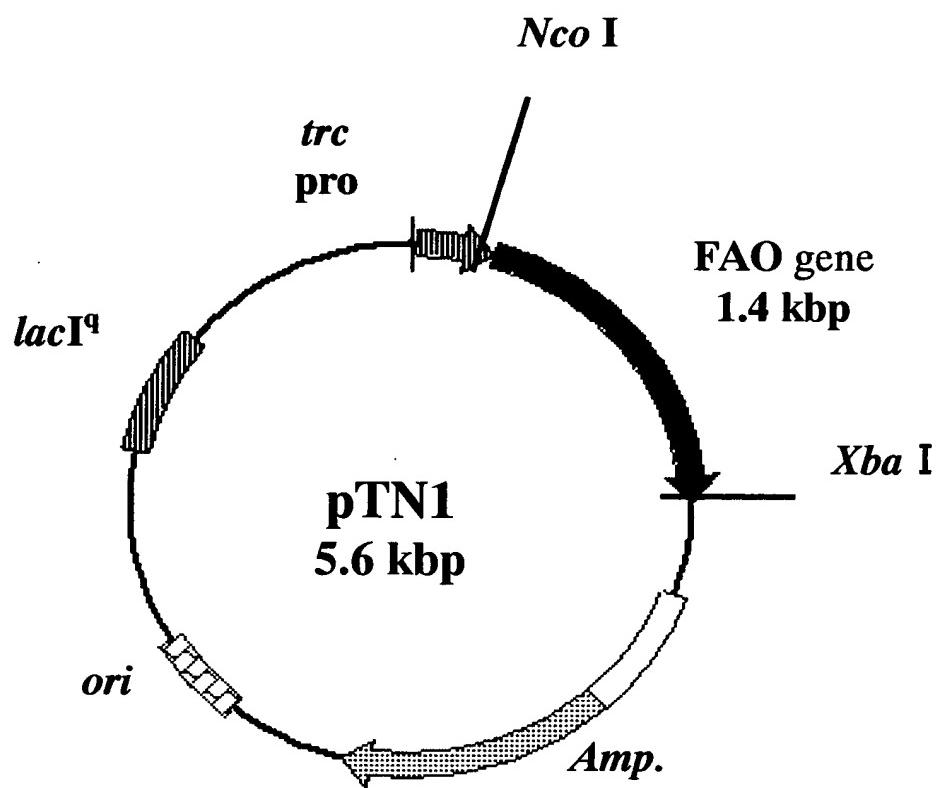


FIG. 5

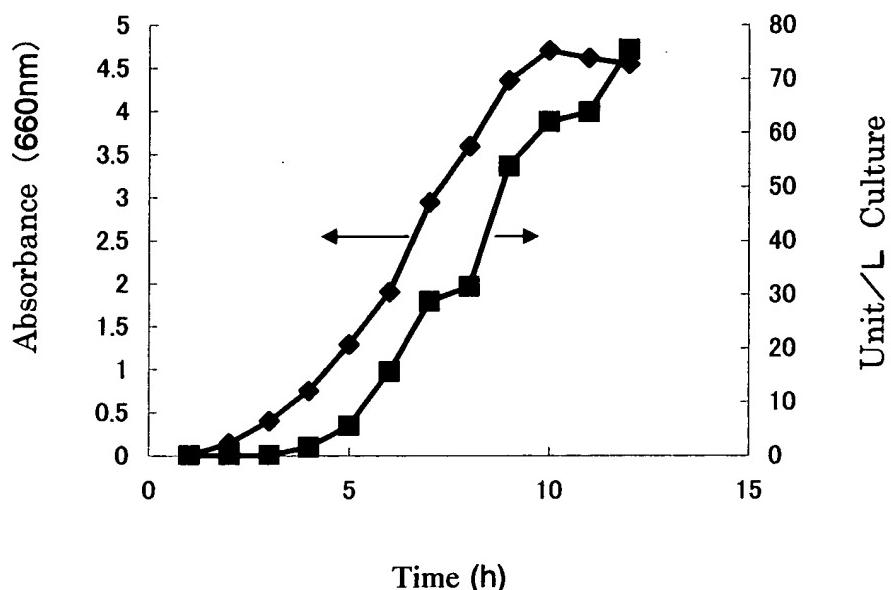
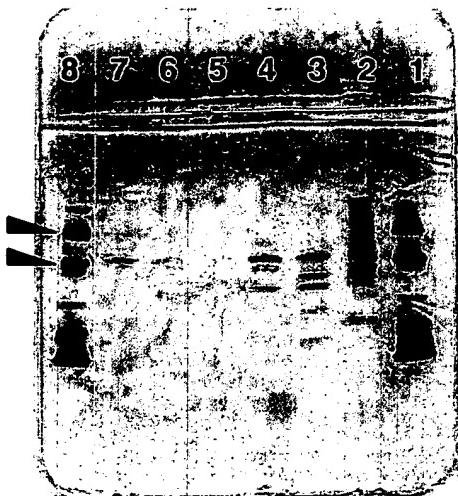


FIG. 6

67kDa
43kDak



- 1: LMW
- 2: Soluble
- 3: after DEAE 5PW
- 4: after RESOURCE Phe
- 5: after Bioassist Q No.23
- 6: after Bioassist Q No.24
- 7: after Bioassist Q No.25
- 8: LMW

FIG. 7

Kinetic parameters

Recombinant	
<i>Km</i> (mM)	5.9
<i>Vmax</i> (U/mg)	7.1

Substrate specificity

Substrate	Activity(%)	
	Recombinant	Wild type
fructosyl valine	100	100
fructosyl lysine	120	135
fructosyl glycine	4	9
fructosyl alanine	56	60
fructosyl leusine	14	31
fructosyl phenylalanine	104	103

FIG. 8